BIOASSAY WITH MAGNETIC MICROSPHERES IN FLOW: A METHOD FOR HIGHLY PARALLEL MOLECULAR SEPARATIONS OF COMPLEX BIOLOGICAL SYSTEMS

Michelle Espy, Christopher Carr, Susan Daniels, Christina Hanson, Andrei Matlachov, Henrik Sandin, and Robert Kraus, Jr.
P-21, Biological and Quantum Physics Group, Physics Division

Steve Graves and John Martin
B-2, Bioscience Division

Mike Ward
MST-11, Materials Science Division

Diandra Leslie-Pelecky
Physics Department, University of Nebraska, Lincoln

A variation on conventional flow cytometry:

- Conventional FCM relies on fluorescent microspheres to label targets
- Binding with analytes also produces fluorescence
- Two lasers interrogate
- Limited by colors of microspheres (10 – 100)

This method relies on replacing fluorescent microspheres with magnetic ones

- Applications

Drug discovery, molecular targeting, DNA analysis, proteomics, and understanding the pathways of cell cycle regulation.

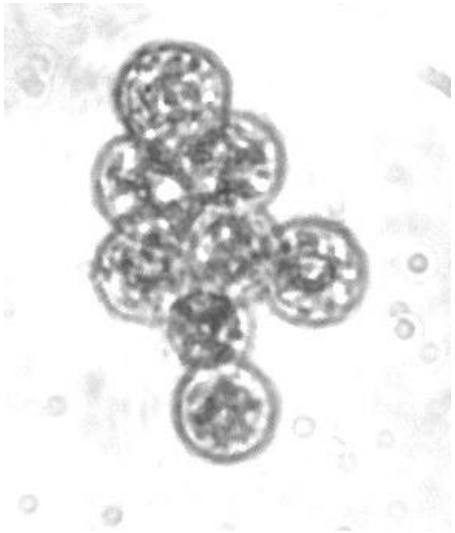
Combining SQUIDs for target identification with laser diagnostics to assess binding provides an efficient, high throughput multiplexed bioassay method based on traditional flow cytometry.

This work is funded by an NIH grant that started August 15th, 2005

The grant was submitted May 2003 and recommended for funding March 2004.

-**Magnetic beads**

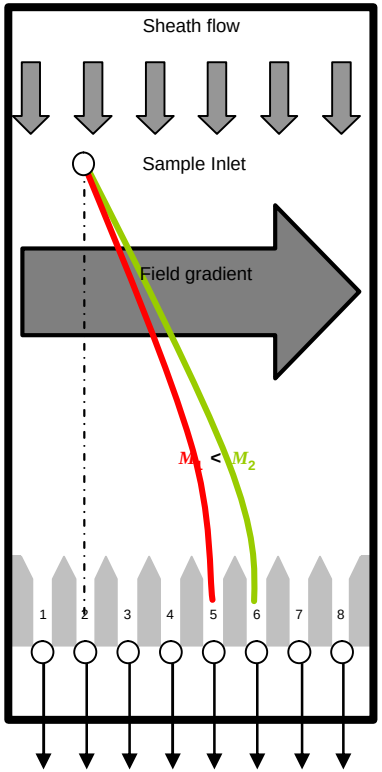
Magnetic microspheres with a range of magnetic moments. Sizes ~ 1-10 μm.
Ideally these would be ferromagnetic with a high remnant magnetization (i.e. SmCo).



1) Magnetic microspheres that will ultimately be suitable for conjugation with target biomolecules will be produced… This milestone will be completed when micrometer sized microspheres consisting of SmCo5 nanoparticles, *or some other suitable material*, encased in polymer have been synthesized and characterized.

Magnetic flow spectrometer for separation

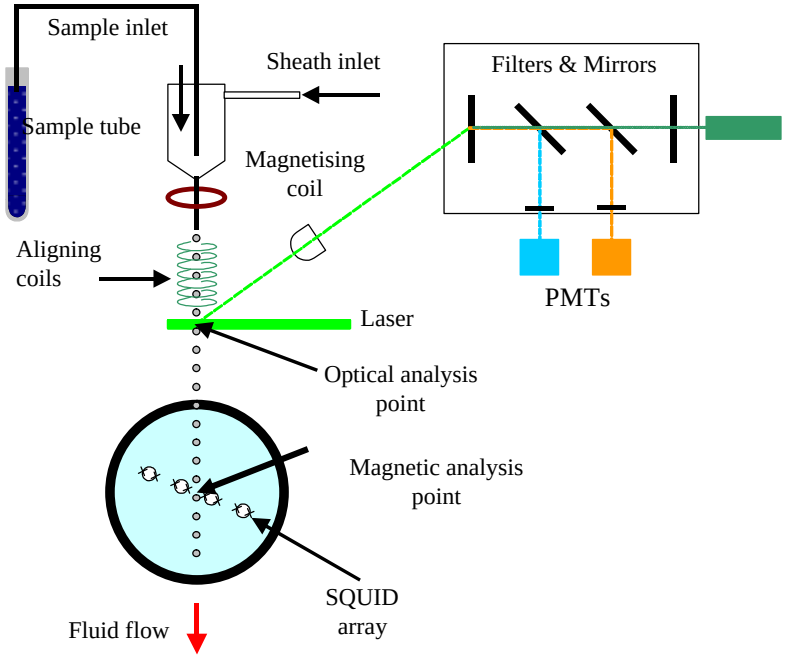
The flow spectrometer system sorts magnetic microspheres by their magnetic moment. This is done in a special chamber subjected to a magnetic field gradient. Sorted magnetic microspheres are functionalized and chemically bound to target molecules so that each species of magnetic moment is bound to a unique kind of molecule.



2) A continuous flow magnetic particle spectrometer capable of parallel and reproducible separation of five populations of magnetically encoded microspheres will be developed. …. After resorting, none of the inappropriate bins can have more than 20% of the total beads collected.

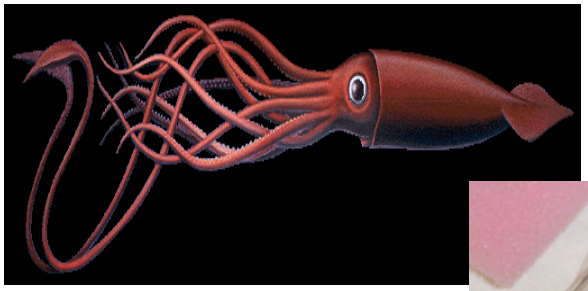
Magnetic flow cytometer for identification

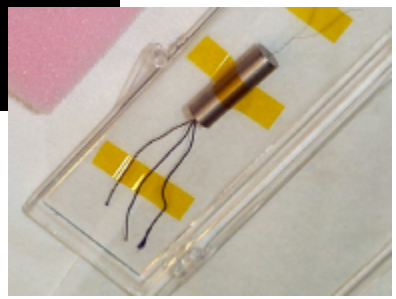
Microspheres are then incubated with analytes and the collection is flowed through a magnetic flow cytometer combining a novel SQUID-based target-molecule tag identification with fluorescence-based analyte detection.

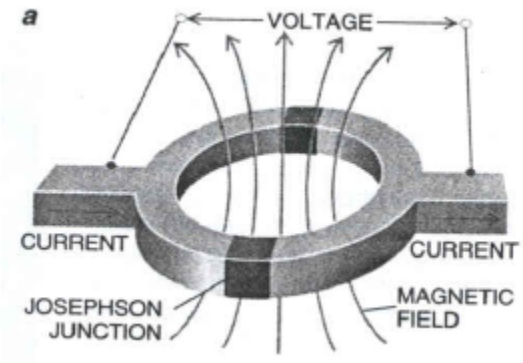


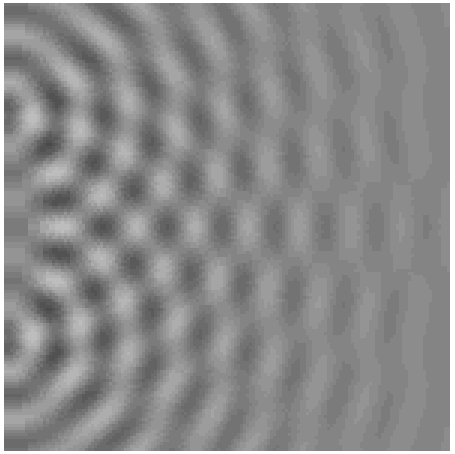
3) A flow cytometer capable of correlated magnetic moment and optical measurements on individual particles will be developed and characterized… Successful completion of this milestone requires the … ability to detect single beads flowing past the SQUID detector and the laser in sequence. The cytometer must detect a single particle for all five species that were separated in milestone 2.

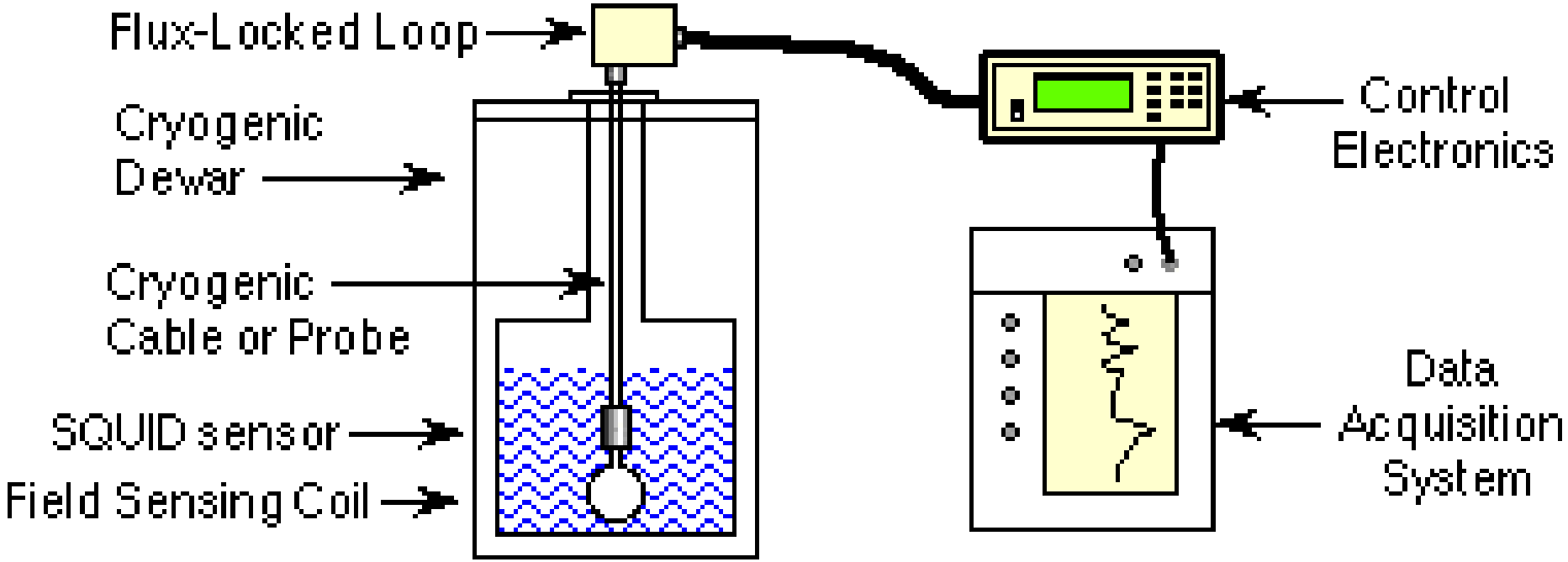
The Superconducting Quantum Interference Device (SQUID) is the world's most sensitive detector of magnetic fields.

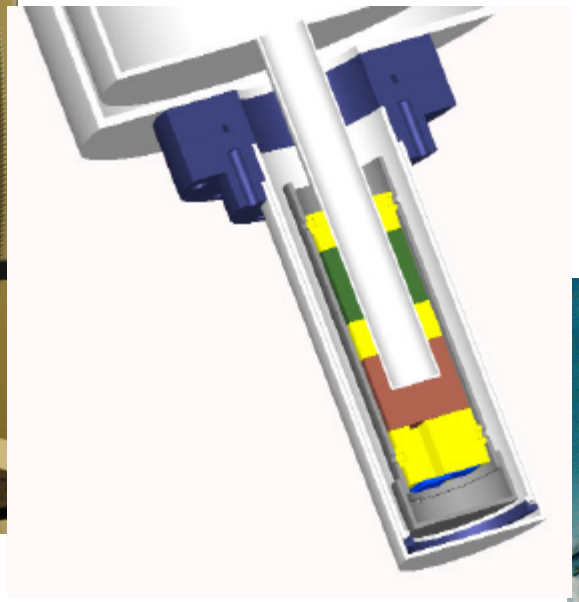


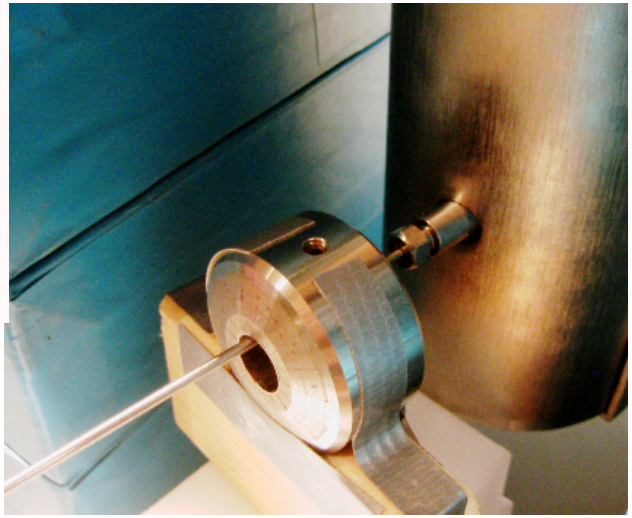




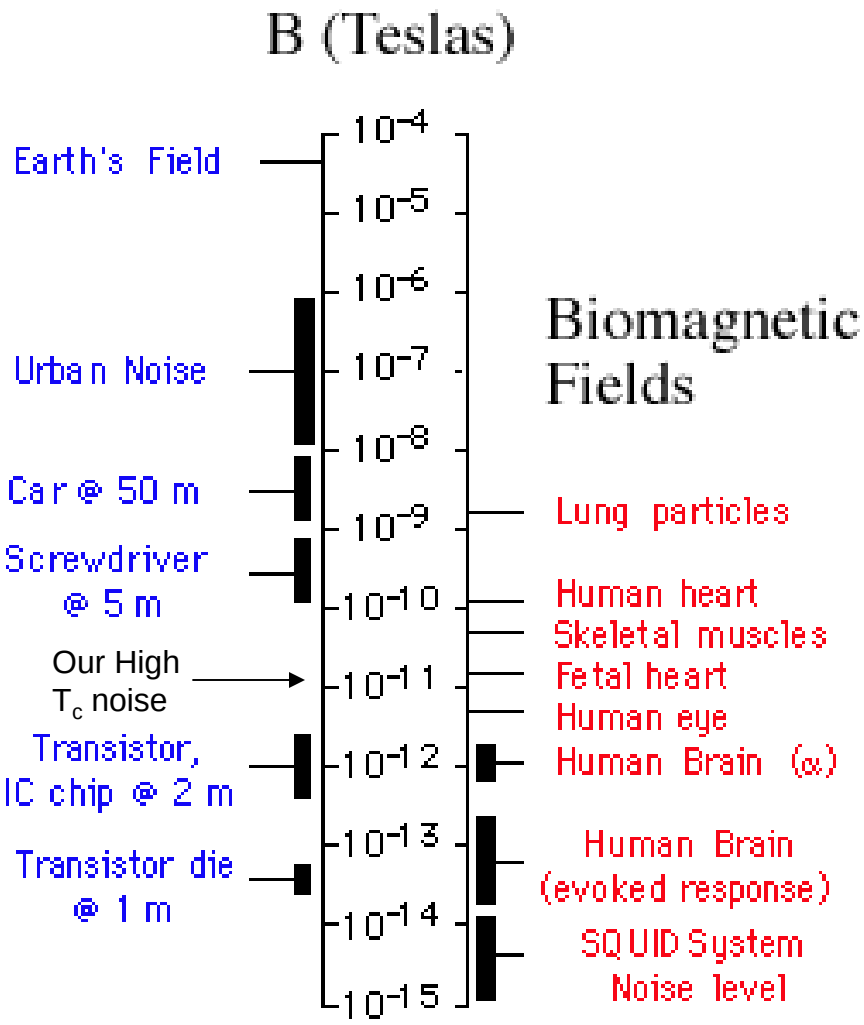


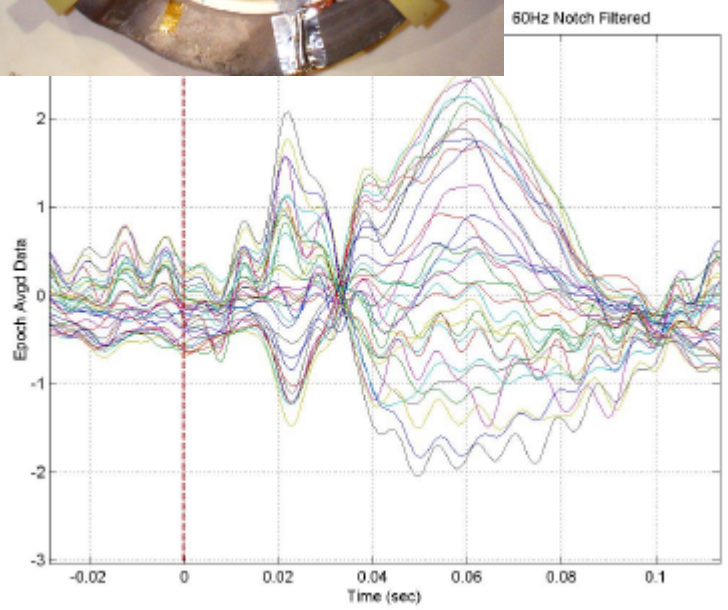






Magnetic Fields





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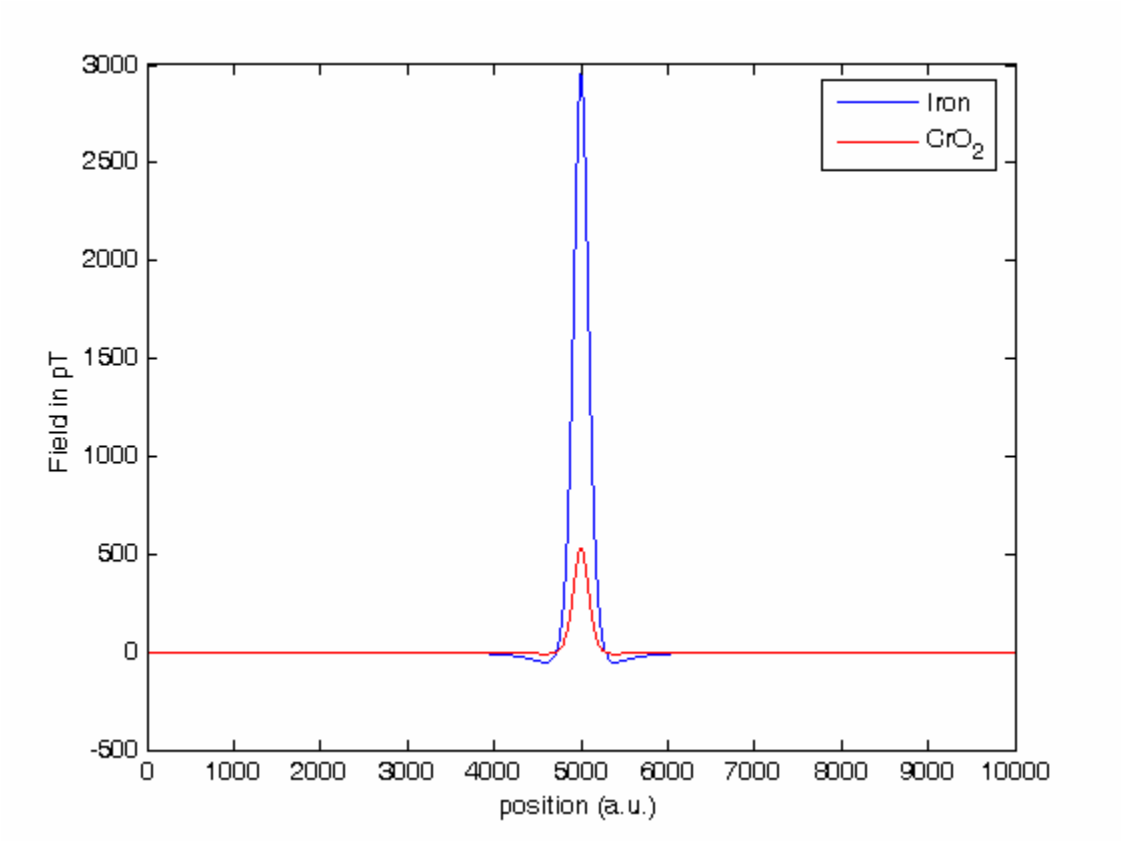


Magnetic beads : Magnetic microspheres with a range of magnetic moments

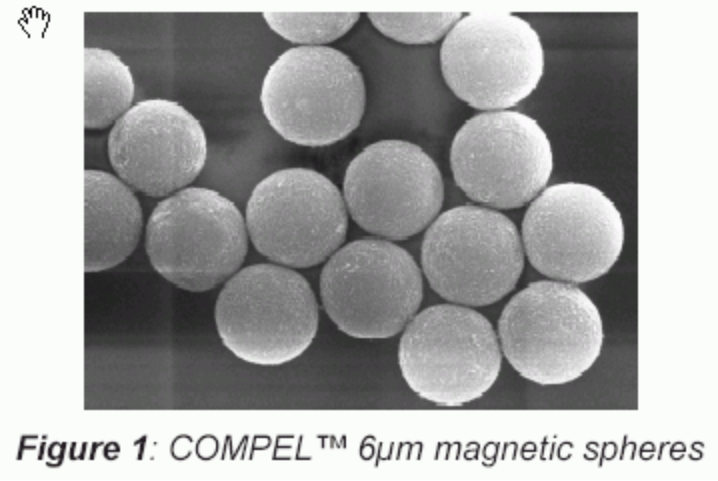
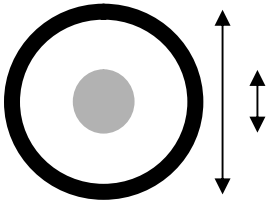


***Figure 1**: COMPEL™ 6µm magnetic spheres*

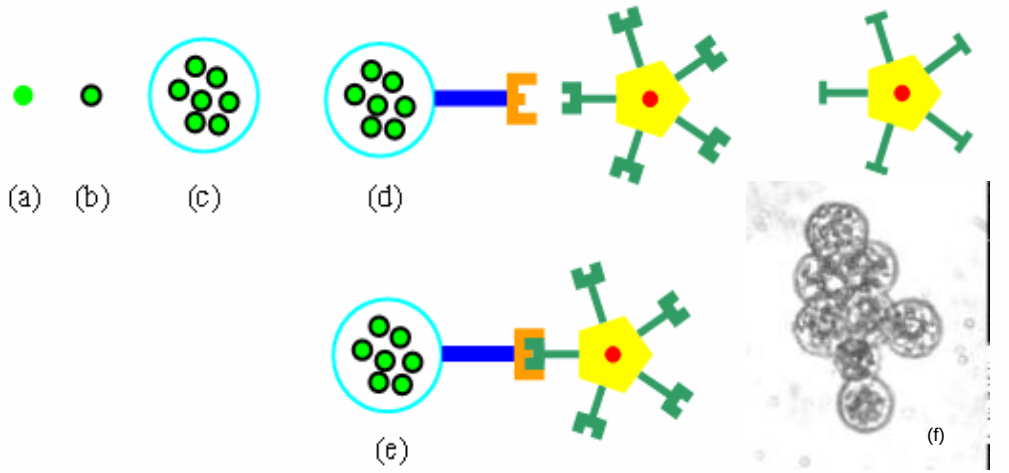
The material must be coated, sorted, and attached to the right agents.

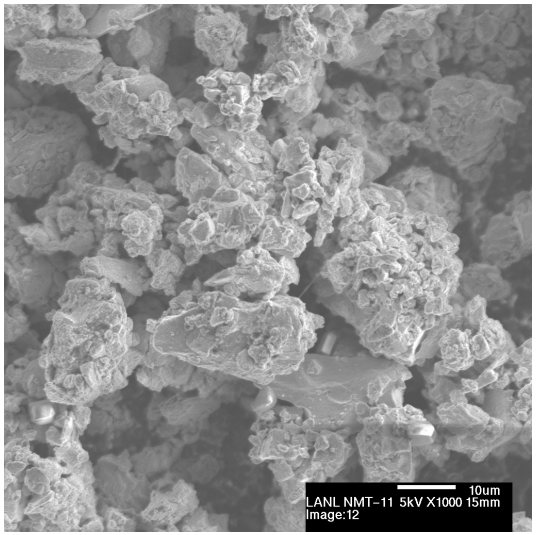


Polymer bead with diameter ~ 1micron

Magnetized core with diameter about ½ that size

We want beads from SmCo: A magnet made of this material is 10 times stronger than your average refrigerator magnet of the same size.





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- We wanted beads from SmCo (high remnance and coercivity) however they oxidized and didn't have stable properties even when coated with carbon

-We have obtained CrO_2 ferromagnetic beads from Spherotech, 4μm diameter. They have about 1/10th the remnance of SmCo.

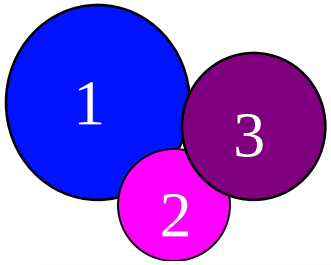
-We have obtained paramagnetic/superparamagnetic samples from:

University of Nebraska (Iron, 54emu/g) variety of sizes 1-100μm

Bangs Labs (Iron Oxide, 2.5emu/g) 8μm

-Paramagnetic/Superparamagnetic particles should (and do) sort just fine – but present challenges for the SQUID portion of the process

Magnetic flow spectrometer for separation: The flow spectrometer system sorts magnetic microspheres by their magnetic moment, enabling highly parallel separations.



microspheres are sorted by magnetic moment by flowing through a chamber where a magnetic field gradient induces a force such that they are collected in different bins with narrow distributions of magnetic moment

$$F_{drag} = 6phav$$

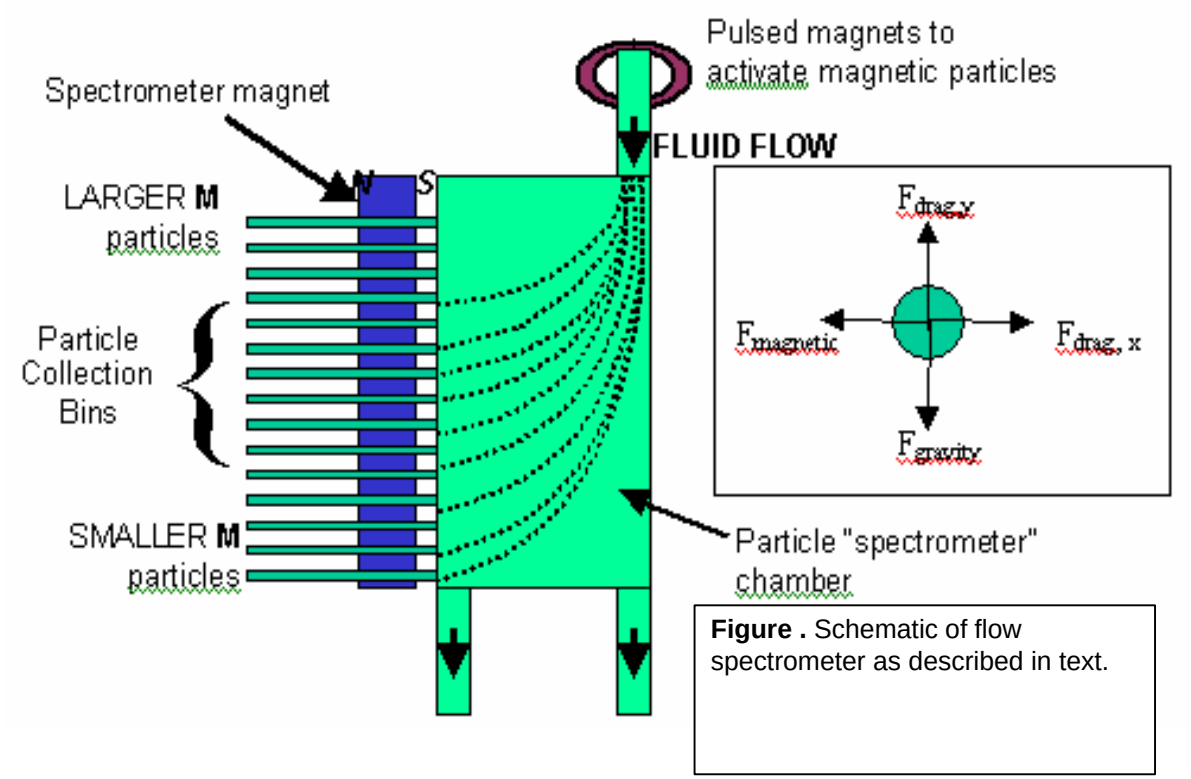
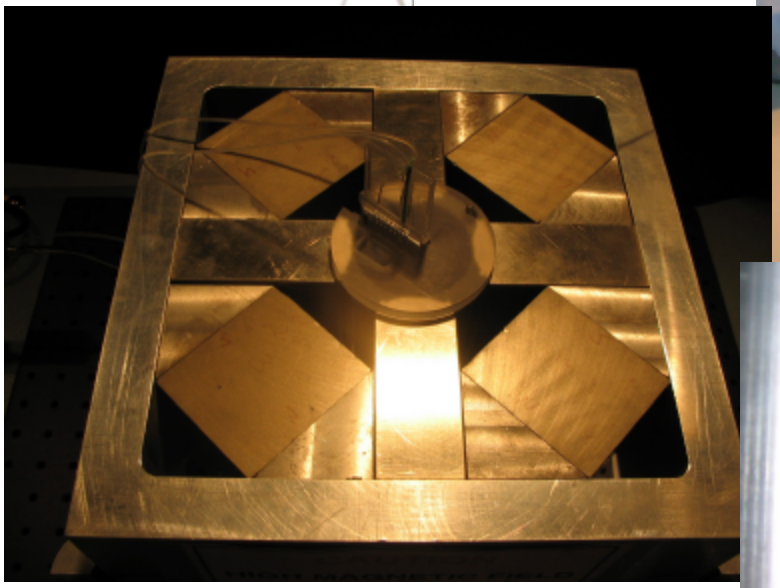
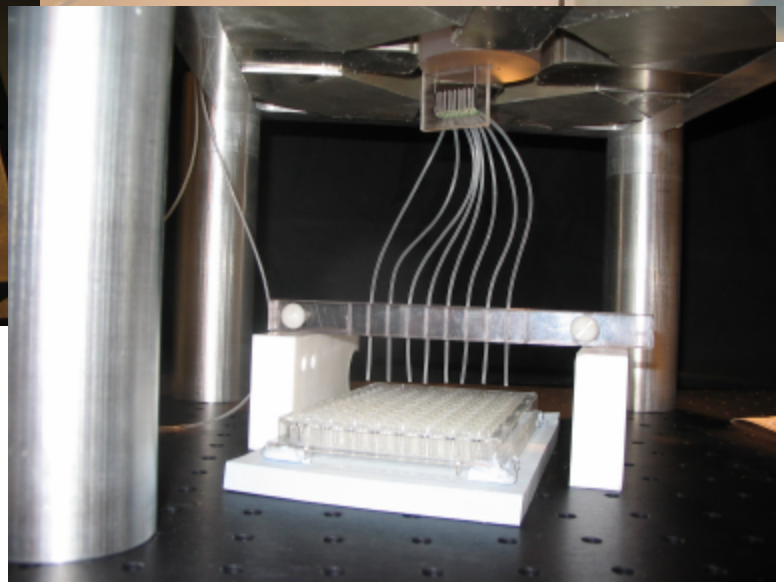


Figure . Schematic of flow spectrometer as described in text.

$$F_{magnetic} = \frac{MVG}{m_0}$$





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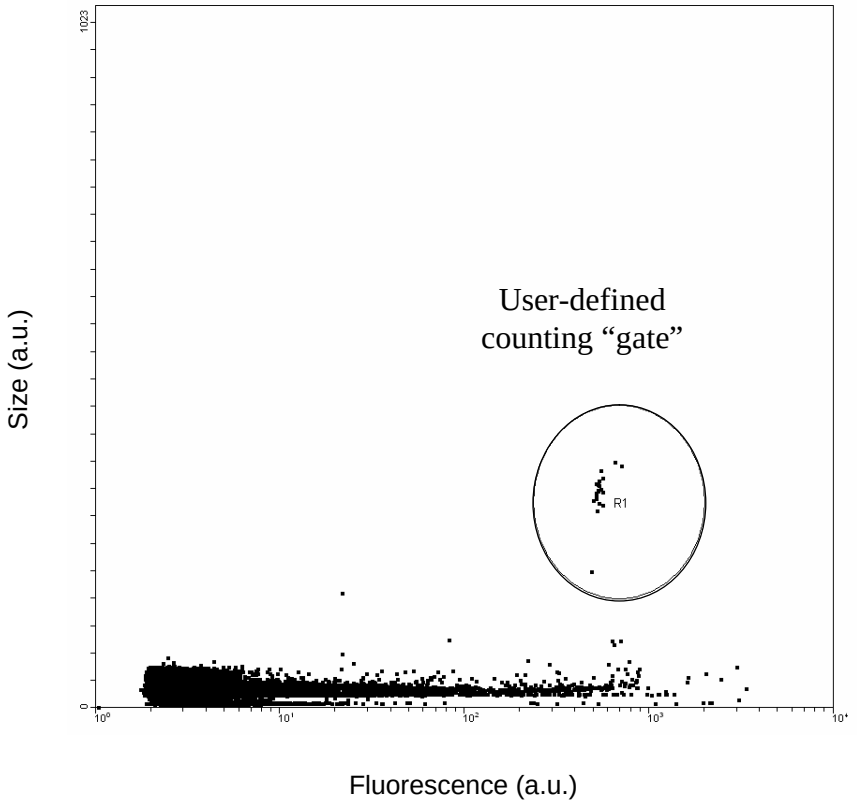

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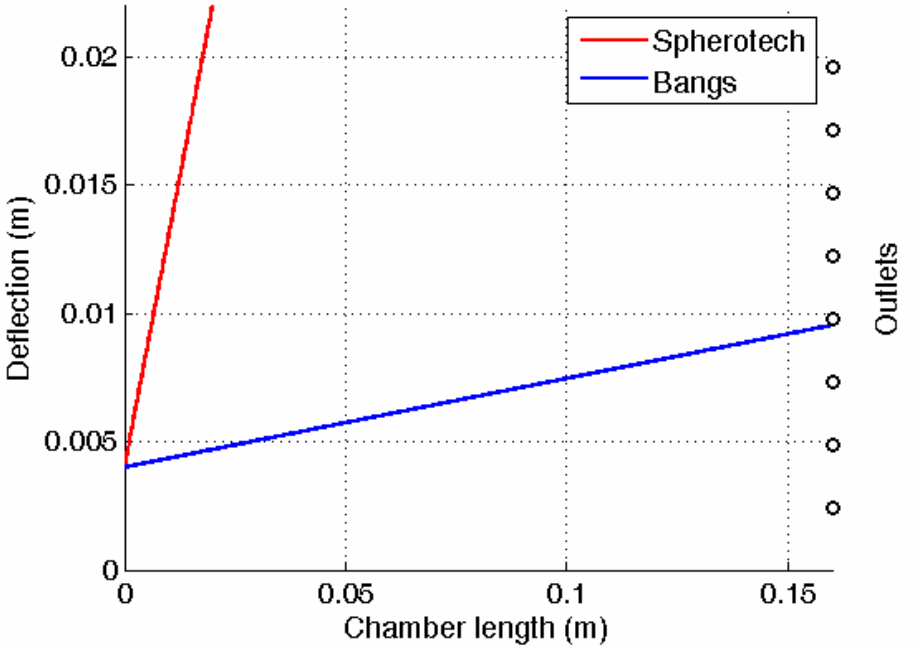

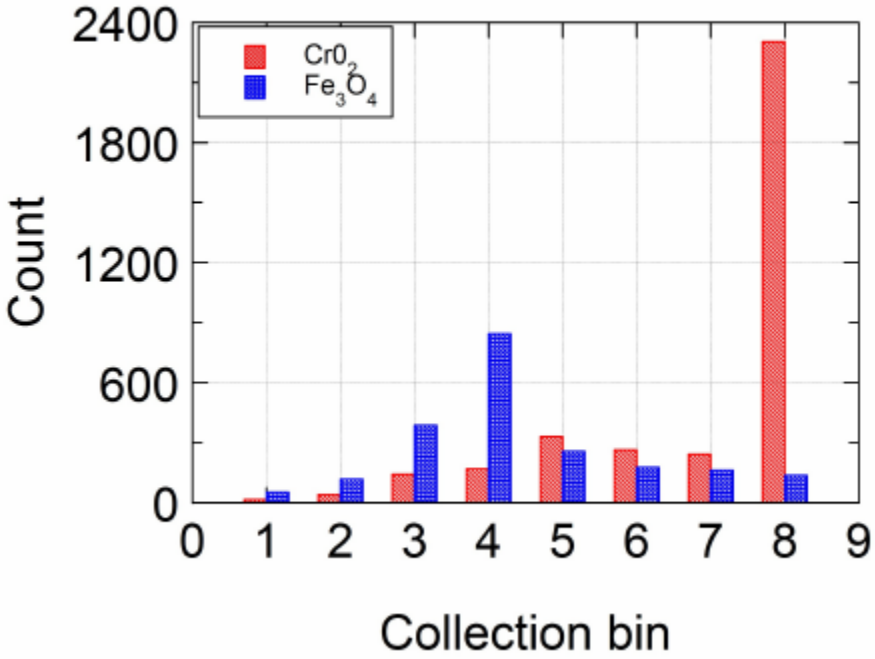

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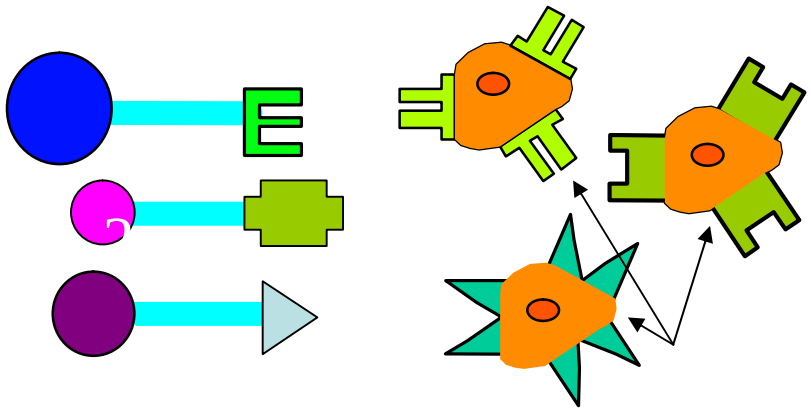
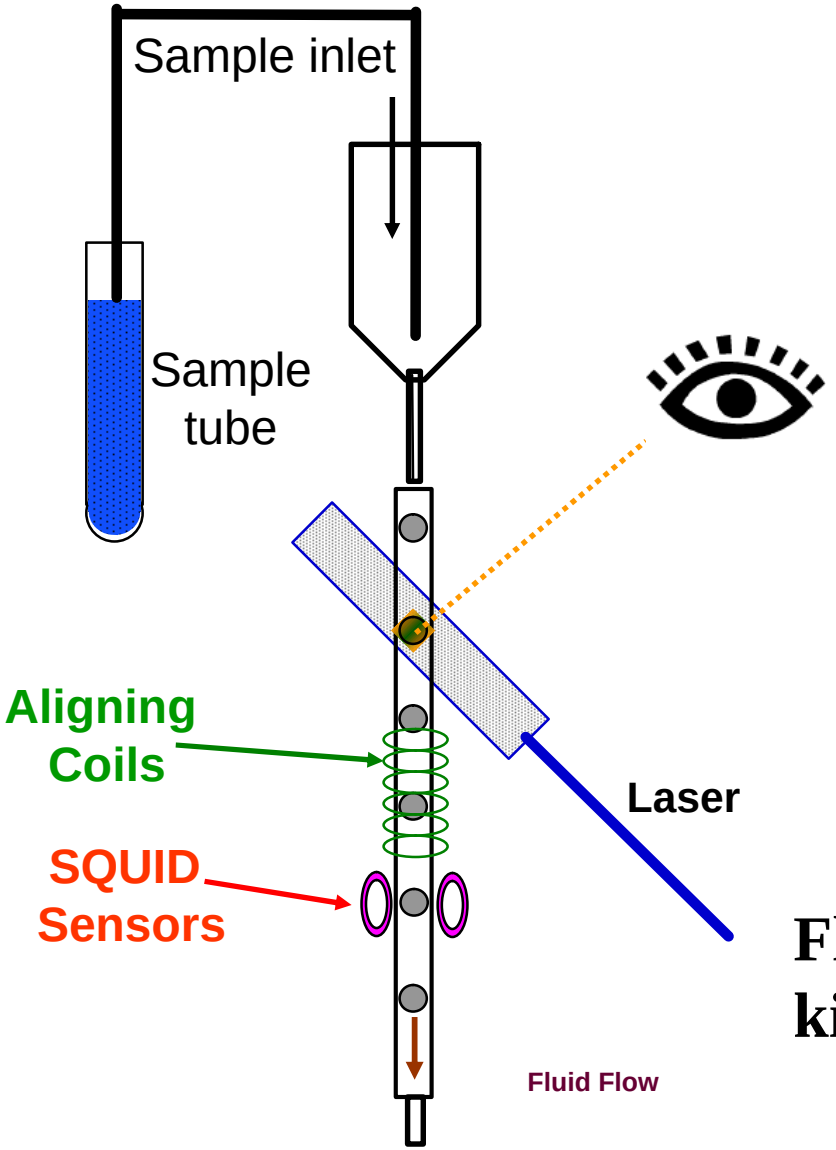
Sorted magnetic microspheres are chemically bound to target molecules so that each species of magnetic moment is bound to a unique kind of molecule. Microspheres are then incubated with analytes



Magnetic flow cytometer for identification: The collection is flowed through a magnetic flow cytometer combining a novel SQUID-based target-molecule tag identification with fluorescence-based analyte detection.



Illuminated by laser, fluorescence tells you there was binding, don't really do this with your eye!

Flows past SQUID to tell you what kind of label

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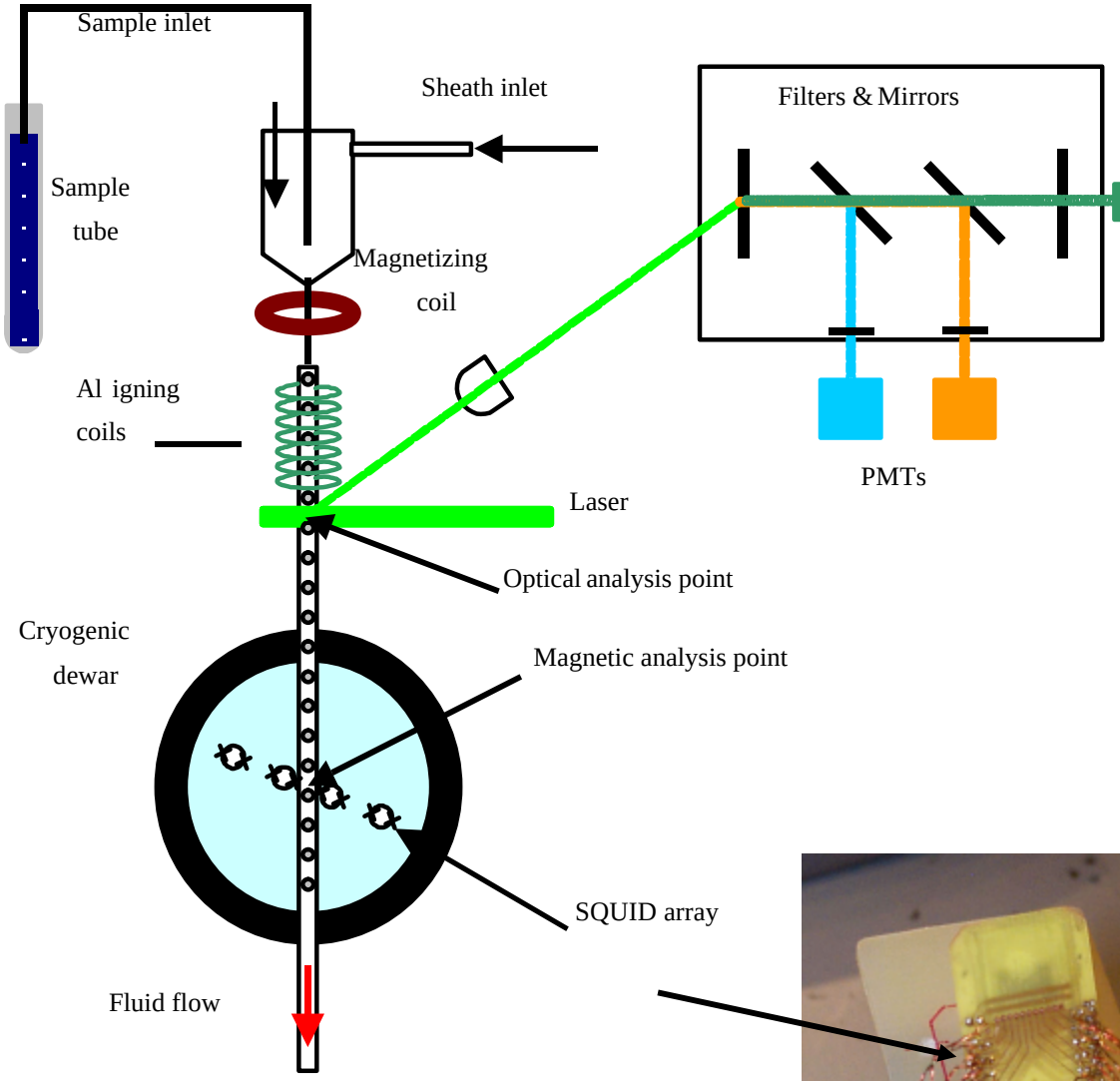
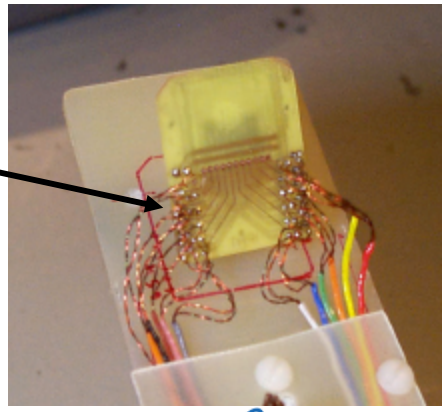
The SQUID detector system identifies the target molecule by measuring the magnetic moment of the microsphere to which it is attached. The cryogenic dewar has a specially designed tail showing where particles enter and pass ~1mm from the SQUID. The optical flow cell for laser interrogation is just outside the dewar tail.





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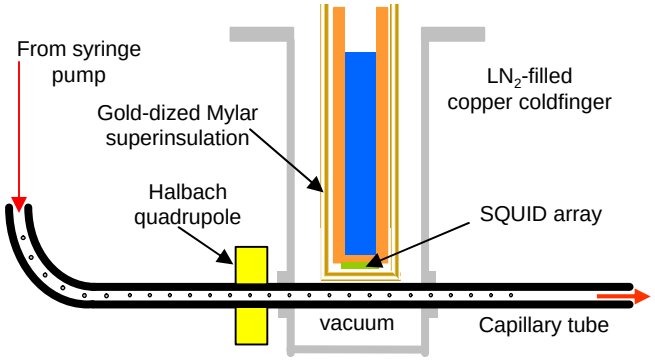
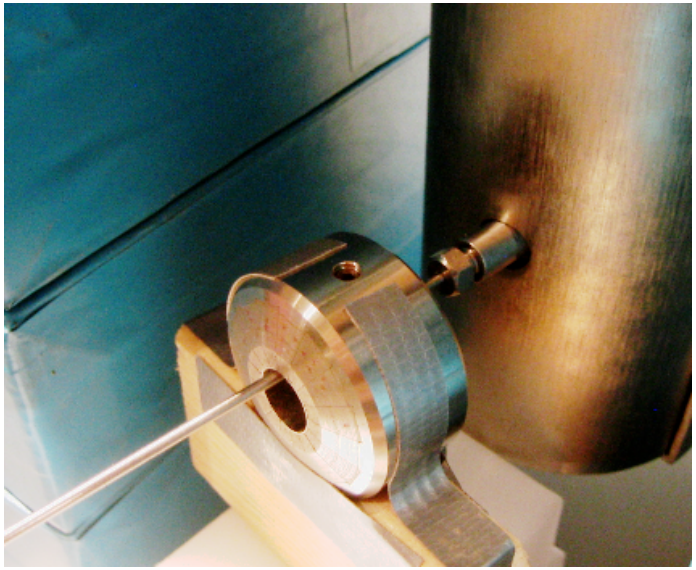
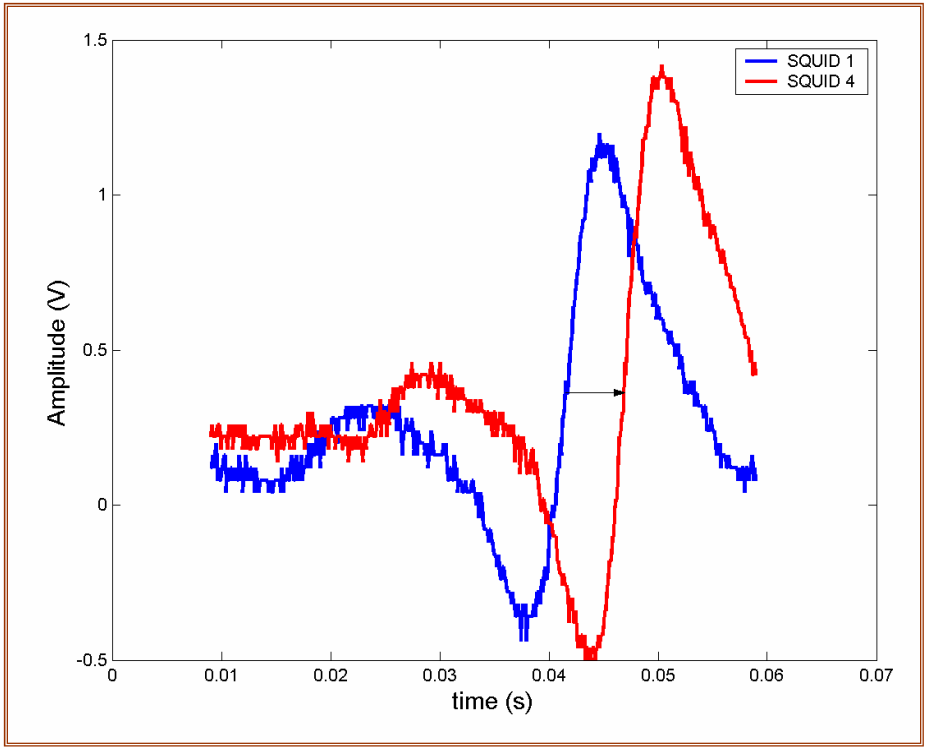
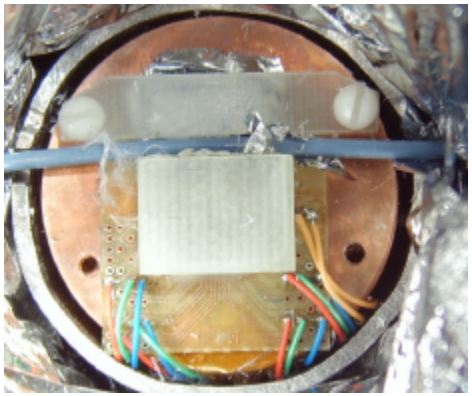
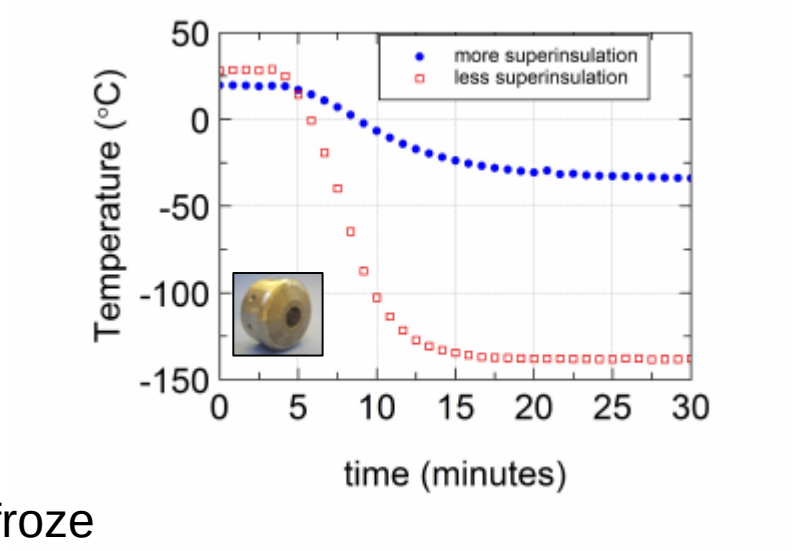






Signal from a bead passing under two SQUIDs. By knowing the separation and the timing shift we can calculate that the bead is traveling about 0.5 m/sec.



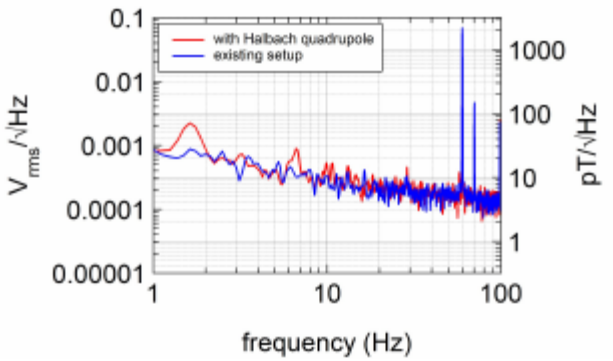


When we switched the tube to plastic it froze

Back to Titanium tube and problem resolved

Because particles are paramagnetic we need the magnet – but so far we still are losing signal due to relaxation

Lift-off needs to be better (~2mm at present)



Future improvements:

Add a solenoid around cold finger

Add thermocouple

Bigger (more sensitive SQUIDs)

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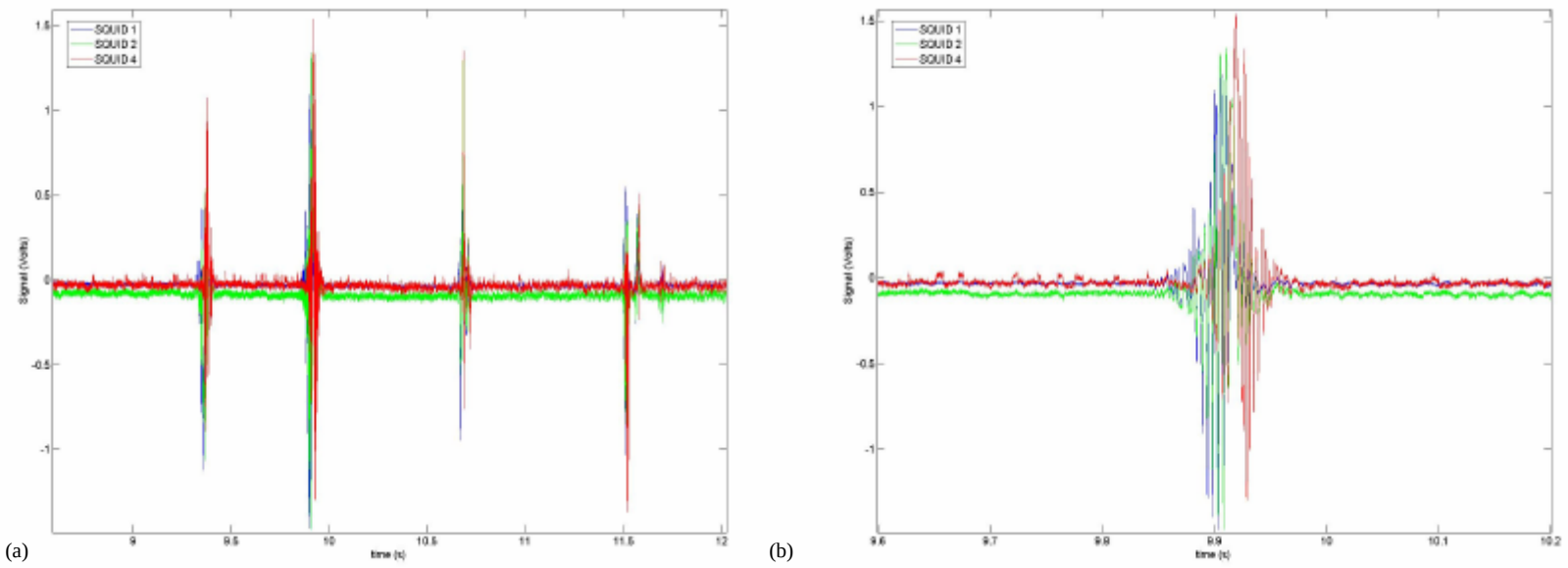
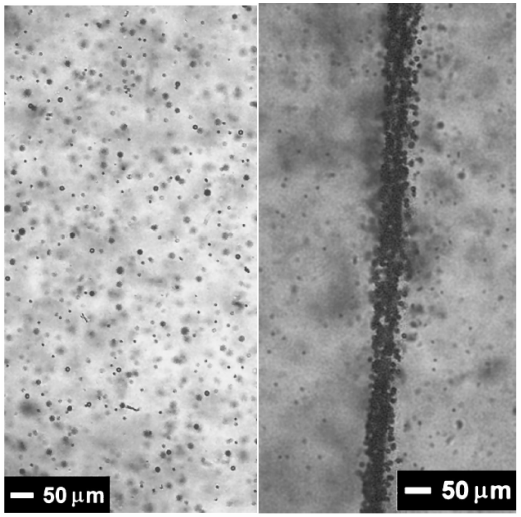


The response of 3 SQUIDs in the linear array to SmCo5 flakes. In (b) a more detailed view is give of 2nd event from the left, showing the flake passing under SQUIDs 1, 2 & 4 in that order.



Accoustic focusing of particles

*G. Goddard and G Kaduchak, J. Acoust. Soc. Am., **117**, 3440, 2005.

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CONCLUSIONS

- A new approach to massively parallel biomolecular assay and separations based on magnetic and fluorescent labeling of microparticles is being developed.
- Work on encapsulation of magnetic nanoparticles of suitable magnetic material and properties is ongoing
- We have built and are demonstrating the hydrodynamic and sorting properties of the flow spectrometer
- We have demonstrated detection of magnetic microspheres using SQUID sensors – but need to push to smaller particle size and more regulated flow
- We have previously demonstrated simultaneous magnetic and optical detection – but only with very large particles and not in timed coincidence